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Activation of the Unfolded Protein Response in Vitiligo: The Missing Link?

Thierry Passeron[1,2] and Jean-Paul Ortonne[1]

Vitiligo is characterized by a substantial loss of functional melanocytes in the epidermis and sometimes in hair follicles. Genetic and pathophysiological studies have provided strong evidence that vitiligo is a polygenetic, multifactorial disorder. The key roles of oxidative stress within melanocytes and anti-melanocyte immune responses have been addressed in many studies, but the relationship between these mechanisms remains unclear. In this issue, Toosi *et al.* report the upregulation of IL-6 and IL-8 after the activation of the unfolded protein response (UPR) following exposure of melanocytes to phenols. Their results shed light on the missing link between oxidative stress and immune responses in vitiligo.

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Oxidative stress in vitiligo

Vitiligo melanocytes long have been known to have a low replication rate in culture, requiring catalase supplementation for their growth and showing structural aberrations, such as dilated endoplasmic reticulum (Boissy *et al.*, 1991). High levels of hydrogen peroxide have been found in the vitiligo epidermis (Schallreuter *et al.*, 1999). Indeed, vitiligo melanocytes are the theater of a redox balance loss because of high levels of toxic catechol metabolites, high free-radical levels, and defective antioxidant machinery (Dell'anna and Picardo, 2006). In culture, vitiligo melanocytes are highly susceptible to chemical and physical oxidative stress. The overactivation of some sources of reactive oxygen species (ROS), the role of NADPH oxidase in ROS formation caused by extracellular and intracellular factors/conditions, and deficiencies in one or more of the reducing agents and detoxifying enzymes are responsible for this redox homeostasis impairment. Dysfunction of catalase, superoxide dismutase, glutathione peroxidase, thioredoxin peroxidase, thioredoxin reductase, and γ-glutamyl transpeptidase has been reported extensively for vitiligo. The first evidence supporting the concept that stressed melanocytes could activate an immune response was provided by the identification of activated dendritic cells after intrinsic damage to melanocytes due to 4-tertiary butyl phenol exposure (Kroll *et al.*, 2005).

Vitiligo, an autoimune disorder

Studies of the infiltrate in perilesional epidermis of vitiligo skin reveal predominantly CD8+ cells (Lili *et al.*, 2012). A recent mouse model of vitiligo, with a phenotype similar to that observed in humans, was developed using melanocyte-specific CD8+ T cells (Harris *et al.*, 2012). Interestingly, in this model, the inhibition of IFN-γ with antibody prevents the development of depigmentation. Th1 and, more recently, the Th17 cytokine environment have been implicated in the pathophysiology of vitiligo (Wang *et al.*, 2011; Kotobuki *et al.*, 2012). Dermal dendritic cells with activated inflammasomes have been found at the edges of vitiligo lesions, and they could participate in the Th17 activation (Wang

[1]*Department of Dermatology, University Hospital of Nice, France and* [2]*INSERM U1065 Team 12, Nice, France*

Correspondence: Thierry Passeron, Department of Dermatology, Archet 2 Hospital, CHU Nice, 151, Route Saint-Antoine de Ginestière, 06200 Nice, France. E-mail: passeron@unice.fr

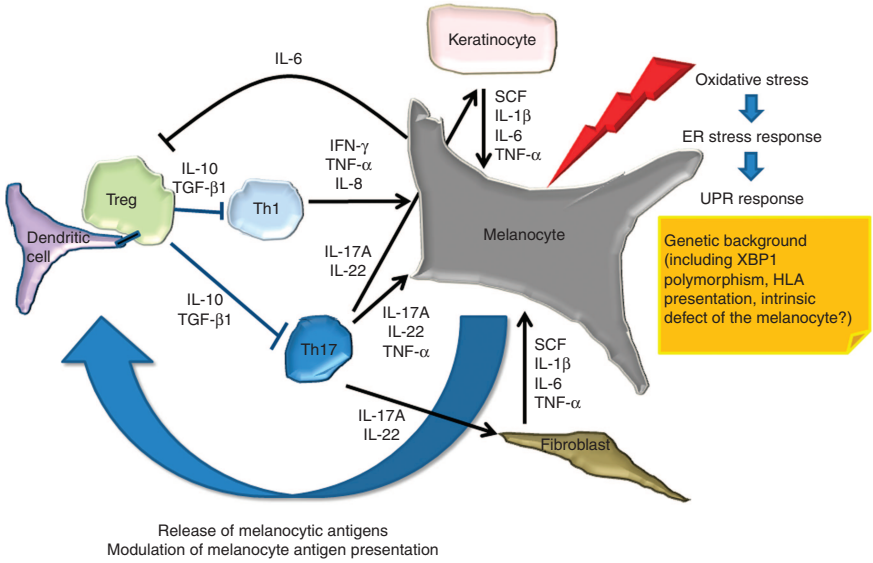

Figure 1. Scheme of the interplay among factors involved in vitiligo pathophysiology. ER, endoplasmic reticulum; SCF, stem cell factor; TGF-β1, transforming growth factor-β; TNF-α, tumor necrosis factor-α; Treg, regulatory T cells; UPR, unfolded protein response; XBP1, X-box binding protein 1.

et al., 2011). Regulatory T cells (Tregs) modulate Th1 and Th17 responses, and there is growing evidence supporting their key role in many immune disorders. Tregs were recently shown to be reduced in vitiligo skin (Klarquist *et al.*, 2010). Some data have also suggested impaired function of Tregs that is associated with a widespread activation of cytotoxic T cells, in vitiligo (Lili *et al.*, 2012). Interestingly, in psoriasis, IL-6 allows cytotoxic T cells to escape from Treg suppression and promotes Th17 differentiation through Stat3 phosphorylation (Goodman *et al.*, 2009). The upregulation of IL-6 production by melanocytes under oxidative stress through the activation of the UPR (Toosi *et al.*, 2012) could thus reduce Treg modulation, leading to the activation of the immune response against melanocytes at a distance from initially affected sites.

Genetic background

Vitiligo is a polygenetic disorder characterized by incomplete penetrance, multiple susceptibility loci, and genetic heterogeneity associated with non-genetic factors. Genetic association studies with candidate genes and, more recently, genome-wide linkage studies of multiplex generalized vitiligo families have revealed several susceptibility genes. Some candidates are melanocytic genes (*TYR*, MC1R, OCA2-HERC2), whereas others (HLA class I, II and III, *NLRP1, PTPN22, XBP1, SMOC2, FOXP1, FOXP3, CCR6, CTLA4, LPP, TSLP, IL2RA, UBASH3A, C1QTNF6, RERE, GZMB, IFIH1, CD80, CLNK, BACH2, SLA, CASP7, CD44, IKZF4, SH2B3, TOB2*) are involved in immune regulation and/or are associated with other autoimmune disorders (Spritz, 2012). Allelic variations in these genes are not sufficient to develop vitiligo, as a concordance of only 23% was found in monozygotic twins (Alkhateeb *et al.*, 2003). Environmental triggers thus appear mandatory at least to initiate the immune process. In this respect, we hypothesized that the activation of XBP1 in response to the oxidative stress induced by phenols, as reported in this issue by Toosi *et al.* (2012), could be enhanced in subjects with specific allelic variations in the *XBP1* gene. Variations in other candidate genes involved in immune regulation could act in the same way in affected individuals to initiate an immune "runaway". Interestingly, some variants of *TYR* (S192Y and R402Q) have recently been shown to modulate the amount of TYR antigen presentation by HLA-A*02:01 (Jin *et al.*, 2012). These variants are associated with an increased risk of vitiligo, whereas HLA-A*02:01 is also associated with a relatively good response to melanoma immunotherapy. Monobenzone exposition was also shown to confer potent immunogenicity. Ubiquitination of tyrosinase protein by monobenzone exposure enhances the presentation of this enzyme by HLA class I and the secretion of melanocyte antigens in response to monobenzone-induced ROS (van den Boorn *et al.*, 2011). These data suggest that modulation in the presentation of melanocyte antigens could also affect the deregulated immune responses against melanocytes in patients.

> Vitiligo pathogenesis includes interplay among oxidative stress and immunity.

A complex network with many cellular factors involved

Aside from the well-demonstrated role of immune cells in vitiligo pathophysiology, cytokines produced by keratinocytes and fibroblasts, such as stem cell factor (SCF) and tumor necrosis factor-α (TNF-α), also seem to have roles in the disappearance of melanocytes (Lan *et al.*, 2009). Keratinocyte dysfunction, with increased TNF-α and IL-6 production, has been reported in vitiligo patients (Moretti *et al.*, 2009). Interestingly, tacrolimus ointment, an effective treatment for vitiligo, was shown to decrease TNF-α and to increase IL-10 (a Th1 and Th17 suppressive cytokine) within treated sites (Grimes *et al.*, 2004; Taher *et al.*, 2009). SCF is secreted by keratinocytes and fibroblasts. The expression of SCF and its receptor, cKIT, is decreased in lesional and perilesional vitiligo skin (Kitamura *et al.*, 2004), and vitiligo-like depigmentation has been reported in relation to tyrosine kinase inhibitors such as imatinib, which act on the cKIT pathway (Legros *et al.*, 2005). Last but not the least, intrinsic defects in melanocytes probably have a crucial role in the pathophysiology of vitiligo. Membrane lipid defects in vitiligo melanocytes have been reported, and they are responsible for the generation of reactive oxygen species (Dell'Anna *et al.*, 2010). It would be interesting to know whether this type of intrinsic oxidative stress also activates the UPR and

upregulates IL-6 and IL-8, thus resuming the pathway implicated with exposure to phenols. We could hypothesize that subtle defects in melanocyte catabolism, in defense against oxidative stress, or in melanocyte antigen presentation, affecting only a subgroup of melanocytes during embryogenesis, could, later in life, trigger an immune response against only those melanocytes. This could explain the systematized lesions observed in the segmental forms of vitiligo. A scheme summarizing those interplays is proposed in Figure 1.

Vitiligo is not limited to melanocytes, even though their disappearance is responsible for the clinical phenotype. Upregulation of IL-6 and IL-8 following activation of the UPR after oxidative stress provides a clear link between oxidative stress and the immune response. A better understanding of these mechanisms could provide new targets to prevent or treat patients with vitiligo.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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